Article

Antioxidant Activity, Total Phenolic and Flavonoid Content and LC–MS Profiling of Leaves Extracts of *Alstonia angustiloba*

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Abstract: Plants have a wide range of active compounds crucial in treating various diseases. Most people consume plants and herbals as an alternative medicine to improve their health and abilities. *A. angustiloba* extract showed antinematodal activity against *Bursaphelenchus xylophilus*, antitrypanosomal action against *Trypanosoma brucei* and anti-plasmodial activity against the chloroquine-resistant *Plasmodium falciparum* K1 strain. Moreover, it has demonstrated growth inhibitory properties towards several human cancer cell lines, such as MDA-MB-231, SKOV-3, HeLa, KB cells and A431. DPPH and ABTS assays were carried out to determine the antioxidant activity of the aqueous and 60% methanolic extract of *A. angustiloba* leaves. Moreover, total phenolic and flavonoid contents were quantified. The presence of potential active compounds was then screened using liquid chromatography coupled with a Q-TOF mass spectrometer (LC–MS) equipped with a dual electrospray ionisation (ESI) source. The EC_{50} values measured by DPPH for the 60% methanolic and aqueous extracts of *A. angustiloba* leaves were 80.38 and 94.11 µg/mL, respectively, and for the ABTS assays were 85.80 and 115.43 µg/mL, respectively. The 60% methanolic extract exhibited the highest value of total phenolic and total flavonoid (382.53 ± 15.00 mg GAE/g and 23.45 ± 1.04 mg QE/g), while the aqueous extract had the least value (301.17 ± 3.49 mg GAE/g and 9.73 ± 1.76 mg QE/g). The LC–MS analysis revealed the presence of 103 and 140 compounds in the aqueous and 60% methanolic extract, respectively. It consists of phenolic acids, flavonoids, alkaloids, amino acids, glycosides, alkaloids, etc. It can be concluded that the therapeutic action of this plant is derived from the presence of various active compounds; however, further research is necessary to determine its efficacy in treating diseases.

Keywords: *Alstonia angustiloba*; total phenolic; total flavonoid; antioxidant; LC–MS

1. Introduction

Nowadays, consumer trends show a growing desire towards natural alternatives, such as plants, instead of synthetic products. Interestingly, plants and plant-based products contain naturally occurring phytochemicals, such as phenols, flavonoids, alkaloids, glycosides, lignins and tannins [1]. These phytochemicals have attracted considerable research interest due to their putative health benefits, such as anticarcinogenic, antiatherogenic, antiulcer, antithrombotic, anti-inflammatory, immunological modulating, antibacterial, vasodilatory and analgesic properties [2].

Moreover, plants serve as the primary sources of antioxidants, with the most common natural antioxidants being polyphenols (e.g., flavonoids, phenolic acids, stilbenes, lignans and anthocyanin), carotenoids (e.g., carotenes and xanthophylls) and vitamins (e.g., vitamin C and E) [3]. These antioxidants are essential to reduce the risk of degenerative illnesses by inhibiting or delaying oxidative damage in the cells by scavenging free radicals, such

as peroxide or hydroperoxide [4]. Besides that, natural antioxidants are finding many applications in the food industry, cosmetics industry and pharmaceutical sector as effective counteragents [5].

The bioactive compounds of plant extract exert this antioxidant activity [6] and can be extracted using various solvents, such as methanol, ethanol, acetone and water [7]. Due to the presence of various antioxidant chemicals with varying polarity and chemical properties, which affect their solubility in the solvent, the nature of the extraction solvent has a significant impact on both the extraction yields and the antioxidant capabilities [8]. Water is the most commonly used solvent in the food and pharmaceutical industries due to its low cost, non-toxicity and environmental friendliness. Nevertheless, aqueous organic solvents extract bioactive chemicals from plant materials more effectively than water [9].

Alstonia angustiloba belongs to the Apocynaceae family. The Apocynaceae family has over 250 genera and 2000 species of tropical shrubs, vines and trees [10,11]. It is locally named "pulai" or "pulai bukit" [12,13] and can be spotted on Africa's and Asia's tropical continents [14]. *A. angustiloba* is a medium-sized tropical tree that can reach a height of 45 metres. Their flower is bisexual, with a histellous calyx and a glabrous corolla on the outer portion. At the same time, numerous and fine secondary veins are observed on the elliptical, subacuminate or obtuse leaves (Figure 1), which have stout petioles in whorls of eight to sixteen centimetres long [15].

Figure 1. Fresh leaf of *A. angustiloba* before drying and extraction process.

The most common compound found in the *A. angustiloba* is indole alkaloids. According to Goh et al. (1997), this plant found in the lowland forests of Sabah, Malaysia contains bioactive alkaloids that can be isolated from its bark and leaves [16]. There are 20 alkaloids present in the bark of the stem of *A. angustiloba*, including angustilobine and andranginine. The cytotoxicity of these substances against KB cells has been demonstrated [17].

Recently, the aqueous leaves extract of *A. angustiloba* was reported to inhibit the growth of skin squamous cell carcinoma (A431 cell line) via the activation of the apoptosis mechanism and cell cycle arrest [18]. The antiproliferative effect was also observed in the growth of HeLa, SKOV-3 and MDA-MB-231 cell lines [19]. Furthermore, this plant showed antiplasmodial activity against the K1 strain of *Plasmodium falciparum* [19,20], antinematodal activity against *Bursaphelenchus xylophilus* [21] and antitrypanosomal action against *Trypanosoma brucei brucei* strain BS221 [22].

Scientific information on *A. angustiloba* is scarce, although it has many potential advantages, especially in treating various diseases. Therefore, the present study was carried out to profile the phytochemical substances in the aqueous extract of *A. angustiloba* leaves, particularly the phenolic and flavonoid compounds.

2. Materials and Methods

2.1. Plant Materials

All the leaves of the *A. angustiloba* plant were sampled in May 2019 from the Rimba Ilmu Botanical Garden located in Kuala Lumpur, Malaysia. A voucher specimen (KLU50198) was authenticated and deposited in the herbarium of Universiti Malaya, Kuala Lumpur, Malaysia.

2.2. Preparation of Aqueous Extracts

The leaves of *A. angustiloba* were cleaned in distilled water and dried in a 50 °C oven for five days. The dried leaves were finely ground with an electric blender. Fifteen grams of powdered finest leaves was added to 150 mL of double distilled water before boiling for 20 min. The leaves mixture was filtered using Whatman no. 1 filter paper to obtain a clear filtrate extract solution. The filtrate was then freeze-dried to obtain the powder and stored at −20 °C until further use [18].

2.3. Methanol Extraction by Soxhlet Technique

A Soxhlet apparatus was filled with powdered *A. angustiloba* leaves (250 g). Extraction was performed for 4 h using 1000 mL of 60% methanol (water: methanol, 40:60 *v/v*). Then, the filtration and drying process of suspension was carried out using a rotary evaporator (R-200; BUCHI, Flawil, Switzerland) coupled with a Buchi Vac V-500 pump [23].

$$\text{Percentage of extract yield} = \frac{\text{Weight of extract obtained after extraction}}{\text{Weight of dried leaves before extraction}} \times 100$$

2.4. Antioxidant Activities

(a) 2,2-diphenyl-1-picrylhydrazyl (DPPH)

DPPH radical scavenging was used to measure the antioxidant capacity by referring to Ismail et al. [24], with slight modifications. Briefly, 0.6 mM DPPH stock solution was prepared by mixing 6 mg of DPPH with 25 mL of methanol. For the DPPH working solution preparation, the DPPH stock solution was dissolved in methanol until the absorbance reading reached 1.1 ± 0.02 at 517 nm. In 96-well plates, 50 μL of *A. angustiloba* leaves extract ranging from 12.5 to 800 μg/mL was mixed with 100 μL of DPPH working solution. The mixtures were incubated for 30 min in the dark. The spectrophotometer was used to measure the absorbance at 517 nm. As a positive control, Trolox in concentrations ranging from 12.5 to 800 μg/mL was utilised. The experiments were carried out in triplicate. The inhibition ratio was calculated as the percentage of inhibition using the following formula: percentage inhibition (%) = ((absorbance of control − absorbance of test sample)/absorbance of control) × 100%. The extract concentration providing the half-maximal effective concentration (EC_{50}) was calculated using a graph by plotting the percentage of DPPH radical scavenging activity against extract concentration. The data were presented as mean values ± standard deviation (SD).

(b) 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS)

The ABTS radical cation decolourisation assay described by Ismail et al. [24] was performed. The ABTS radical cation working solution was prepared by mixing 7.5 mM ABTS stock solution with 3.8 mM potassium persulfate. The mixture was then kept at the darkroom temperature for 16 h to obtain a dark-coloured solution containing $ABTS^{·+}$ radicals. The ABTS radical cation working solution was then diluted with methanol for an initial absorbance of approximately 0.70 ± 0.02 at 734 nm. Next, 10 μL of the *A. angustiloba* leaves extract ranging from 12.5 to 800 μg/mL was added to 90 μL of ABTS radical cation working solution in 96-well plates before incubating in the dark. Each assay must be run together with the appropriate solvent blanks. The absorbance was measured at 734 nm using a spectrophotometer and compared to the Trolox control (in the range of 12.5–800 μg/mL). The EC_{50} of ABTS radicals scavenged was used to measure the scavenging activity. The assay was carried out in triplicate, and the results were shown as mean values ± SD.

2.5. Total Phenolic Content (TPC)

The phenolic content in *A. angustiloba* leaves extract was evaluated using a Folin–Ciocalteu technique reported by Phuyal et al. [1], with appropriate modifications. The *A. angustiloba* leaves extract was diluted with methanol to make a stock solution of 1 mg/mL.

A total of 20 μL of the stock solution was added to 100 μL of 10% Folin in a 96-well plate. The mixture was then left in the darkroom for 5 min. The mixture was incubated for 1 to 2 h after being mixed with 80 μL of 7.5% sodium carbonate ($NaHCO_3$). The absorbance was measured at 760 nm, and the TPC was carried out in triplicate. A standard curve was obtained from varying concentrations (12.5 to 800 μg/mL) of gallic acid (standard solution). The total phenolic content was expressed as a percentage of total gallic acid equivalents per gram extract (mg GAE/g).

2.6. Total Flavonoid Content (TFC)

With slight modification, the total flavonoid content of *A. angustiloba* leaves extract was evaluated using the technique reported by Awang et al. [25]. The mixture of *A. angustiloba* leaves extract (100 μL, 1 mg/mL) and 100 μL of 2% aluminium chloride ($AlCl_3$) was incubated for 10 min. Next, the absorbance was measured at 420 nm using a spectrophotometer. Analysis of TFC was performed in triplicate. A standard curve was generated using varying concentrations (10 to 400 μg/mL) of standard solution (Quercetin). Total flavonoid concentration was calculated as a percentage of total quercetin equivalents per gram of extract (mg QE/g).

2.7. Liquid Chromatography–Mass Spectrometry (LC–MS) Analysis of A. angustiloba Leaves Extracts

The study was carried out using an LC system 1290 (Agilent Technologies series Infinity System LC, Santa Clara, CA, USA) paired with a Q-TOF 6520 (Agilent Technologies, Santa Clara, CA, USA) mass spectrometer equipped with dual electrospray ionisation (ESI) source. The technique is based on the protocol reported by Araujo et al. [26], with slight modification. An Agilent Zorbax Eclipse XDB-C18 (narrow bore 2.1 × 150 mm, 3.5 μm) was selected for the chromatographic separation at 25 °C. With a flow rate of 0.50 mL/min, the mobile phases were 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). The gradient elution was as follows: 0–4 min, 5% B; 5–19 min, 5% B; 20–24 min, 100% B; 25–30 min, 100% B. The extracts were diluted in 50% methanol, and 1 μL of the mixture was injected by an autosampler into the column. The mass spectrometry was run in both positive and negative ESI modes, with the following settings: capillary voltage (VCap), 3500 V (negative) and 4000 V (positive); fragmentor, 125 V; skimmer, 65 V; octapole (OCT 1 RF Vpp), 750 V; the pressure of nebuliser, 45 psi; drying gas temperature, 10 L/min and sheath gas temperature, 300 °C. The mass spectra were recorded by scanning the mass ranging from m/z 100 to 3200 in MS modes. The data were processed by Agilent MassHunter Qualitative Analysis software version B.07.0, which provides a list of possible molecular formulas. MS data, MS/MS fragmentation patterns and molecular formula proposed by MassHunter were compared to literature data and several databases, such as Human Metabolome, ChemSpider and PubChem, for the annotation of the phytochemicals identified in the extract. A maximum error of 8 ppm was accepted.

2.8. Statistical Analysis

A one-way analysis of variance (ANOVA) was performed on the data, followed by Tukey's multiple comparison test ($p \leq 0.05$). The data were statistically analysed using GraphPad Prism version 8. The results are reported as the average of three measurements with the standard deviation.

3. Results and Discussion

The present study showed that the percentage of extract yield in aqueous extraction was 27.76% and 44.06%, as obtained from 60% methanol extraction of the Soxhlet method.

3.1. Antioxidant Activity of A. angustiloba Leaves Extracts

Our study evaluated the *A. angustiloba* leaves extracts' antioxidant activity, which was measured using the DPPH and ABTS assays. Both techniques were employed because

the reagent is significantly more stable and convenient to be used than chromogenic radical reagents [27]. Figure 2 demonstrates EC_{50} of the aqueous and 60% methanolic leaves extracts of *A. angustiloba* to quantify their antioxidant capacity and compare their activities. The EC_{50} is an antioxidant concentration needed to achieve a 50% reduction in free radicals [24]. Samples rich in antioxidant levels are usually expressed as lower EC_{50} values [28–30] . The EC_{50} of 60% methanolic extract of *A. angustiloba* was lower than the aqueous extract of *A. angustiloba*, indicating that 60% methanolic extract had a higher antioxidant activity. It might be due to the high concentration of phenolic, flavonoid, alkaloid and terpenoid components found in this extraction [7].
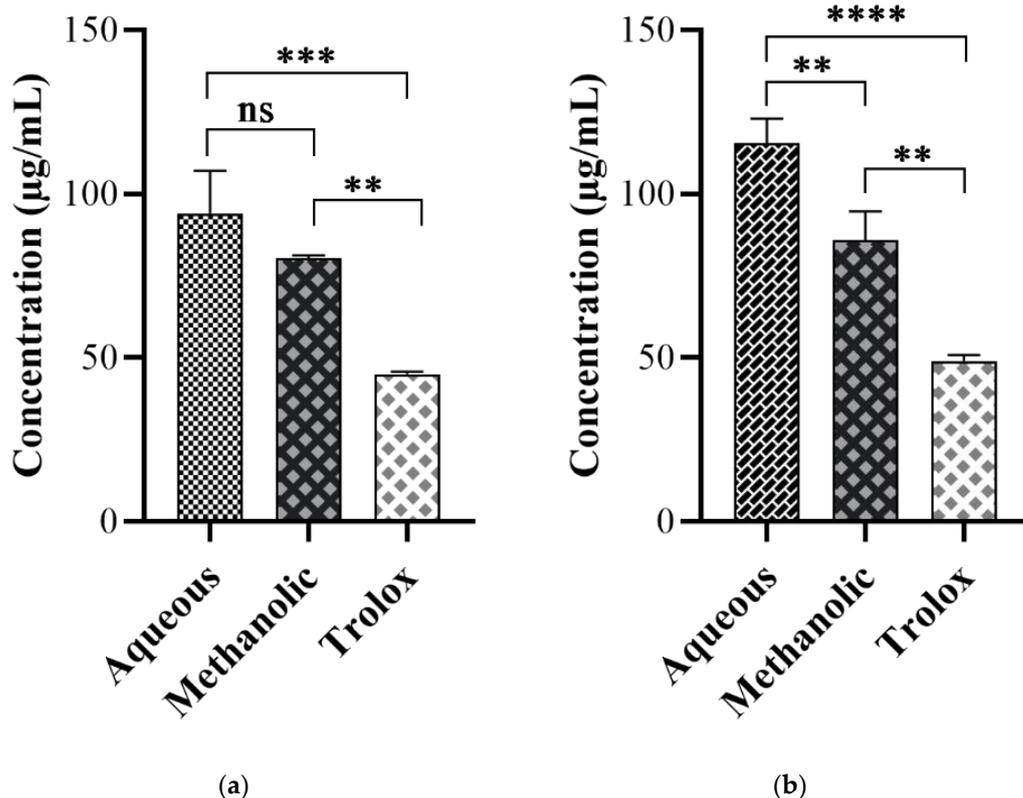

(**a**)   (**b**)

Figure 2. The EC_{50} of *A. angustiloba* leaves extracts for (**a**) DPPH and (**b**) ABTS radical scavenging activities. Data show mean $\pm$ SD, $n = 3$. Values of ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ were considered statistically different & ns is denoted as not statistically significant.

Methanol is a well-known solvent with high polarity properties and can extract substantial amounts of polyphenols compared to water and ethanol. It is believed that greater antioxidant activity correlated with high polyphenol compounds through the synergistic effect of the different polyphenols and the donation of hydrogen atoms [31]. However, methanol usage is frequently questioned due to its toxicity to humans [32].

The active compounds of *A. angustiloba* in the present study were extracted using aqueous (water) and water–methanol (water: methanol, 40:60 *v/v*). Water is usually added to the solvent to improve its polarity, in which the relative polarity of methanol is 0.762 and water is 1 [33], and reduces methanol concentration. Moreover, some studies showed that more polar solvents (aqueous methanol/ethanol) could extract higher amount of - phenolic compounds [34] compared to absolute methanol/ethanol [35]. The EC_{50} measured by DPPH for the 60% methanolic and aqueous extracts of *A. angustiloba* leaves were 80.38 and 94.11 µg/mL, respectively, and, for ABTS assays, the values were 85.80 and 115.43 µg/mL, respectively. The standard used in this study was Trolox, which is a renowned natural antioxidant agent [36]. Trolox shows excellent antioxidant capacity as an established antioxidant, with EC_{50} values obtained from DPPH and ABTS assays being 44.91 and 48.91 µg/mL, respectively. The plant belongs to the same genus of *Alstonia*, such

as *Alstonia parvifolia*, and also possesses a significant capacity for scavenging free radicals with IC$_{50}$: 0.287 mg/mL [37]. Additionally, Akinnawo et al. (2017) reported the aqueous extract of *Alstonia bonnei* was more effective at scavenging DPPH radicals than the other fractions, including ethyl acetate of 70% methanolic extract, hexane and butanol fractions. It could imply that the aqueous extract of *A. boonei* leaves contains antioxidant-rich active compounds [38].

Antioxidants must provide an active hydrogen atom or an electron, thus allowing the antioxidant to scavenge the reactive oxygen species (ROS) [39]. The effective radical scavenging agents, such as flavanol and polyphenol compounds and vitamin C and E, usually contain molecules bearing functional hydroxyl groups [40].

3.2. Total Phenolic and Flavonoid Content of A. angustiloba Leaves Extracts

Polyphenolic compounds are phytochemicals derived from plants [41]. These compounds are organic substances and play a critical role in human health by regulating metabolism, weight, chronic disease and cell proliferation. They have become an emerging area of nutrition in recent years [42].

TPC was estimated by the Folin–Ciocalteu, and TFC was measured by the aluminium chloride method. As shown in Figure 3, the regression equation $y = 0.003x + 0.07$ and an R^2 of 0.9979 were used to calculate the phenolics (TPC) in the extracts. The concentrations obtained were expressed in gallic acid equivalence (mg GAE/g). In contrast, the flavonoid was estimated from the plotted standard curve of quercetin with the regression equation $y = 0.007x + 0.04$ and an R^2 of 0.9980. The concentration obtained was represented in milligrams of quercetin equivalents per gram of the plant extract (mg QE/g).
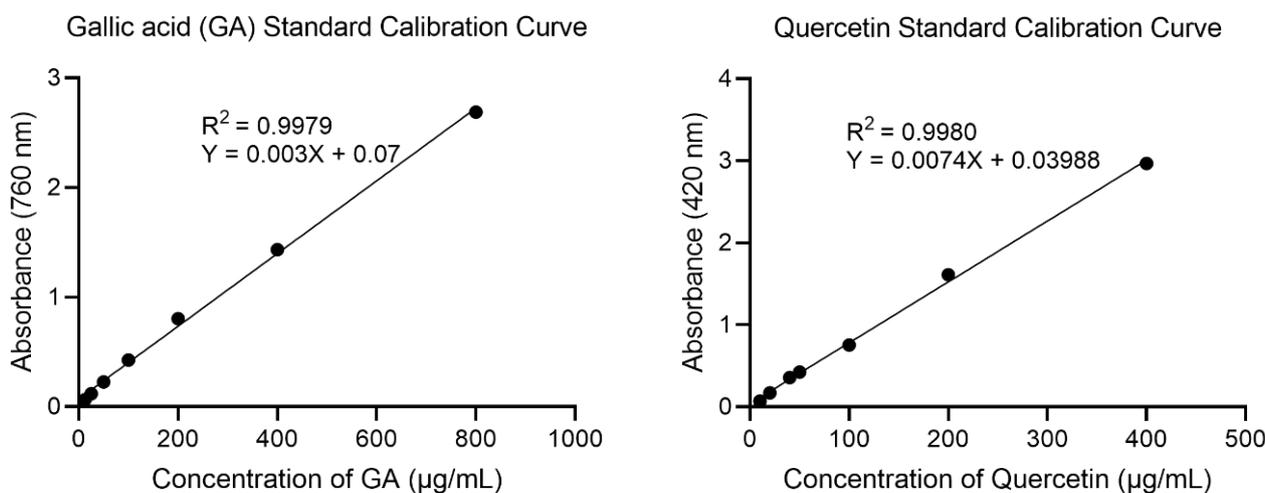
Figure 3. Calibration curve of standards.

Figure 4 represents the data for estimating total phenolic and total flavonoid content in 60% methanolic and aqueous extract of *A. angustiloba* leaves.The TPC and TFC in 60% methanolic extract exhibited the highest value (382.53 $\pm$ 15.00 mg GAE/g and 23.45 $\pm$ 1.04 mg QE/g, respectively), while aqueous extract exhibited the lowest value (301.17 $\pm$ 3.49 mg GAE/g and 9.73 $\pm$ 1.76 mg QE/g, respectively). The plant from the same genus, such as *A. boonei*, has demonstrated a total phenolic acid of 34.13 $\pm$ 1.90 mg GAE/g and a total flavonoid of 19.47 $\pm$ 1.89 mg QE/g [43]. On the other hand, the study reported by Ganjewala and Gupta (2013) indicated the flavonoids and phenolics contents of *A. scholaris* methanolic leaf extract are 97.3 mg QE/g DW and 49.7 mg GAE/g DW, respectively [44].
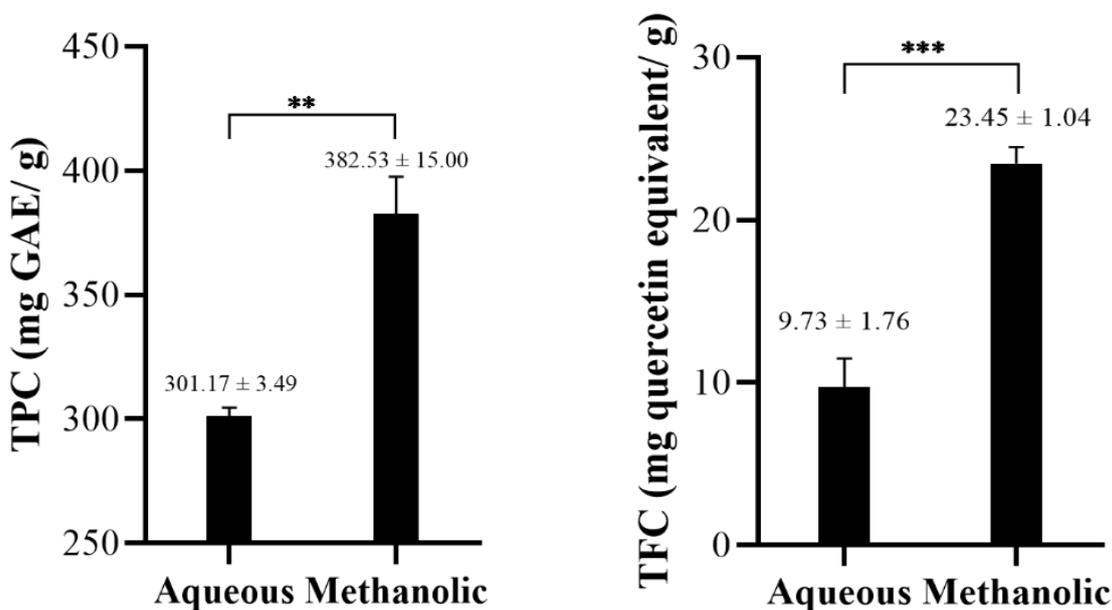
Figure 4. A comparison of TPC and TFC in 60% methanolic and aqueous extract of *A. angustiloba* leaves. Data show mean ± SD, *n* = 3. Values of ** *p* < 0.01, *** *p* < 0.001 were considered statistically different.

3.3. Correlation between Antioxidant Activity and TPC and TFC of A. angustiloba Leaves Extracts

Tables 1 and 2 showed the Pearson's correlation coefficient between the total flavonoid and phenolic contents with activities of antioxidant in the aqueous and 60% methanolic extracts. The antioxidant activity of both extracts showed positive correlations with TPC and TFC. Both extracts showed a significant positive correlation between TPC and DPPH and ABTS scavenging activity, with r = 0.915 (*p* < 0.01) and r = 0.884 (*p* < 0.01), respectively. The findings demonstrated the greater the concentration of flavonoids and phenolics, the greater the antioxidant activity of the extracts.

**Table 1.** Pearson's correlation coefficient of TPC and TFC with antioxidant activity in the aqueous extract of *A. angustiloba*.

	DPPH	ABTS	TPC	TFC
DPPH	1.000	0.958 ***	0.924 **	0.531
ABTS	0.958 ***	1.000	0.891 **	0.406
TPC	0.924 **	0.891 **	1.000	0.775 *
TFC	0.531	0.406	0.775 *	1.000

Significant correlation at * *p* < 0.05, ** *p* < 0.01 and *** *p* < 0.001

Table 2. Pearson's correlation coefficient of TPC and TFC with antioxidant activity in the 60% methanolic extract of *A. angustiloba*.

	DPPH	ABTS	TPC	TFC
DPPH	1.000	0.927 **	0.915 **	0.627
ABTS	0.927 **	1.000	0.884 **	0.449
TPC	0.915 **	0.884 **	1.000	0.808 *
TFC	0.627	0.449	0.808 *	1.000

Significant correlation at * *p* < 0.05 and ** *p* < 0.01.

The variation in phytochemical composition is due to the genetic diversity in different species. Moreover, the discrepancies in results are also found in similar species because plants are strongly determined by environmental factors, such as rainfall, water fluctuation, temperature, humidity, nutrient composition, direct contact with soil microbes and

alteration in soil pH. Environmental factors could interact with the genetics of the plant, resulting in genetic variants and gene regulation [24].

Our findings exhibited that the total flavonoid was lower than total phenolic in both extracts. Previous research demonstrated that antioxidant capacity is strongly associated with total flavonoid and phenolic components of plant leaves' crude extract [24,45]. Phytochemical studies of the genus and species of *Alstonia* sp. are limited, yet the plant has great potential to treat many serious diseases.

3.4. LC–MS Analysis of A. angustiloba Leaves Extracts

LC–MS equipped with a Q-TOF high analyser was employed to evaluate the phytochemicals profile in the aqueous and 60% methanolic extracts. All the annotated compounds are summarised in Tables 3–6 with their retention time (min), *m/z* experimental, teoric mass, MS/MS fragments, molecular formula generated by the MassHunter and error (ppm), as proposed by Araujo et al. (2020) [26].

Table 3. Phytochemical profile of the aqueous extract of *A. angustiloba* leaves by LC–MS in the positive ion mode.

Compound	ts (min)	*m*/z Experimental	Teoric Mass	MS/MS Fragments	Molecular Formula	Error (ppm)
Phenolic acids						
m-Coumaric acid	8.231	165.0543 [M + H]+	164.047	151	C9 H8 O3	1.8
Gallic acid	1.575	171.0286 (M + H)+	170.0214	141/151/160	C7 H6 O5	0.51
Aconitic acid	0.903	175.0232 (M + H)+	174.0161	149/157	C6 H6 O6	1.89
Quinic acid	0.668	193.071 (M + H)+	192.0637	174	C7 H12 O6	−1.84
4-(2-hydroxypropoxy)-3,5-dimethyl-Phenol	9.113	197.1171 (M + H)+	196.1099	-	C11 H16 O3	0.31
3-Methoxy-4,5-methylenedioxybenzoic acid	0.627	219.026 (M + Na)+	196.0369	209	C9 H8 O5	1.65
4-p-Coumaroylquinic acid	8.007	339.1079 (M + H)+	338.1006	-	C16 H18 O8	−1.36
Flavonoids						
5,7,2′,3′-Tetrahydroxyflavone	9.59	287.0552 (M + H)+	286.0481	265/275	C15 H10 O6	−1.29
ent-Fisetinidol-4beta-ol	7.327	291.087 (M + H)+	290.0797	262	C15 H14 O6	−2.14
3,5,7,2′,5′-Pentahydroxyflavone	9.326	303.0505 (M + H)+	302.0431	273/289	C15 H10 O7	−1.5
2′,4′,6′-Trihydroxy-3′-prenyldihydrochalcone	8.976	327.1594 (M + H)+	326.1519	303	C20 H22 O4	−0.15
Isovitexin	9.755	433.1138 (M + H)+	432.1066	-	C21 H20 O10	−2.29
6-C-Galactosylisoscutellarein	9.185	449.1086 (M + H)+	448.1016	434	C21 H20 O11	−2.21
6-Hydroxyluteolin 5-rhamnoside	9.325	449.1091 (M + H)+	448.1017	-	C21 H20 O11	−2.59
8-Hydroxyluteolin 8-glucoside	8.89	465.1035 (M + H)+	464.096	341	C21 H20 O12	−1.13
Apigenin 7-(2″-E-p-coumaroylglucoside	6.935	579.1509 (M + H)+	578.1435	-	C30 H26 O12	−1.81

Compound	ts (min)	*m/z* Experimental	Teoric Mass	MS/MS Fragments	Molecular Formula	Error (ppm)
Flavonoids						
Isovitexin 7-O-rhamnoside	9.591	579.1716 (M + H)+	578.1642	327	C27 H30 O14	−1.12
Luteolin 7-rhamnosyl(1->6)galactoside	9.212	595.1669 (M + H)+	594.1596	449	C27 H30 O15	−1.98
Robinetin 3-rutinoside	8.729	611.16 (M + H)+	610.1527	341	C27 H30 O16	1.11
Robinetinidol-(4alpha->8)-catechin-(6->4alpha)-robinetinidol	7.545	867.2129 (M + H)+	866.2055	420	C45 H38 O18	0.38
Alkaloids						
Gentiatibetine	1.821	166.0859 (M + H)+	165.0787	143/151	C9 H11 N O2	1.97
Fagomine	0.871	170.0795 (M + Na)+	147.0903	147/163	C6 H13 N O3	−5.01
Boschniakine	0.697	184.0726 (M + Na)+	161.0833	163/174	C10 H11 N O	4.53
Sarpagine	8.783	311.1752 (M + H)+	310.1679	289/303	C19 H22 N2 O2	0.71
Quinidine	9.747	325.1922 (M + H)+	324.1849	305/317	C20 H24 N2 O2	−3.38
Yohimbic Acid	8.782	341.1866 (M + H)+	340.1792	-	C20 H24 N2 O3	−1.52
14β-Hydroxyyohimbine	9.861	371.1969 (M + H)+	370.1897	341/352	C21 H26 N2 O4	−1.14
Glycosides						
Scopolin	7.304	355.103 (M + H)+	354.0958	327/337	C16 H18 O9	−2.08
Blumenol C glucoside	9.221	373.2229 (M + H)+	372.2159	355	C19 H32 O7	−3.06
Dihydroferulic acid 4-O-glucuronide	8.2	390.1408 (M + NH$_4$)+	372.1075	351/373	C16 H20 O10	−4.87
(1RS,2RS)-Guaiacylglycerol 1-glucoside	6.886	394.1717 (M + NH$_4$)+	376.1377	-	C16 H24 O10	−1.94
Benzyl O-[arabinofuranosyl-(1->6)-glucoside]	7.644	420.1869 (M + NH$_4$)+	402.1526	390/402	C18 H26 O10	−0.08
Lucuminic acid	8.104	464.1773 (M + NH$_4$)+	446.1434	341	C19 H26 O12	−2.07
Eugenol O-[a-L-Arabinofuranosyl-(1->6)-b-D-glucopyranoside]	9.306	476.2131 (M + NH$_4$)+	458.1791	449	C21 H30 O11	−0.55
Mascaroside	8.977	542.2601 (M + NH$_4$)+	524.2261	465	C26 H36 O11	−0.58
Prupaside	8.581	570.2548 (M + NH$_4$)+	552.2214	540	C27 H36 O12	−1.26
(7'R)-(+)-Lyoniresinol 9'-glucoside	8.404	600.2655 (M + NH$_4$)+	582.2315	570/579	C28 H38 O13	−0.41
Fatty acids						
8S-hydroxy-2E-Decene-4,6-diynoic acid	6.888	179.0702 (M + H)+	178.063	153/167	C10 H10 O3	−0.31
10-Tridecynoic acid	10.447	211.1692 (M + H)+	210.1619	-	C13 H22 O2	0.59
9-keto palmitic acid	11.757	271.2273 (M + H)+	270.2198	253	C16 H30 O3	−1.22
9,16-dihydroxy-palmitic acid	11.758	289.2386 (M + H)+	288.2306	271	C16 H32 O4	−1.91

Table 3. *Cont.*

Compound	ts (min)	*m/z* Experimental	Teoric Mass	MS/MS Fragments	Molecular Formula	Error (ppm)
Others						
Isoamyl nitrite	0.673	118.0864 [M + H]+	117.079	104	C5 H11 N O2	−0.52
Pyroglutamic acid	0.923	130.0498 [M + H]+	129.0425	-	C5 H7 N O3	0.4
Piperonal	7.341	151.0388 [M + H]+	150.0316	121/139	C8 H6 O3	0.83
3-Hydroxycoumarin	7.303	163.039 [M + H]+	162.0319	139/151	C9 H6 O3	−1.38
2-Propenyl propyl disulfide	1.08	166.0723 (M + NH$_4$)+	148.0386	121/149	C6 H12 S2	−3.48
3-tert-Butyl-5-methylcatechol	12.155	181.1221 (M + H)+	180.1148	158	C11 H16 O2	1.36
N-Hydroxy-L-phenylalanine	1.015	182.0809 (M + H)+	181.0737	166	C9 H11 N O3	1.32
3,4-Dehydro-6-hydroxymellein	7.343	193.0492 (M + H)+	192.042	163/171	C10 H8 O4	1.33
2,3-Dihydroxy-p-cumate	6.889	197.0808 (M + H)+	196.0736	167/179	C10 H12 O4	−0.43
N17-Dimethylindole-3-carboxaldehyde	7.882	197.0813 (M + Na)+	174.0921	179	C11 H12 N O	−1.16
2-Phenylethyl 3-methylbutanoate	7.774	207.1376 (M + H)+	206.1304	179/197	C13 H18 O2	1.4
(5alpha,8beta,9beta)-5,9-Epoxy-3,6-megastigmadien-8-ol	10.261	209.1538 (M + H)+	208.1464	183/195	C13 H20 O2	−0.36
Vanilpyruvic acid	7.342	211.0602 (M + H)+	210.0529	193	C10 H10 O5	−0.6
6-(2-Methoxyvinyl)benzo[1,3]dioxole-5-carboxylic acid	9.014	223.0601 (M + H)+	222.053	197/209/219	C11 H10 O5	−0.87
Haematommic Acid, Ethyl Ester,	8.231	225.0757 (M + H)+	224.0686	197/211	C11 H12 O5	−0.42
2-Hydroxy-3-carboxy-6-oxo-7-methylocta-2,4-dienoate	7.344	229.0714 (M + H)+	228.0641	211	C10 H12 O6	4
Depdecin	7.844	229.1073 (M + H)+	228.1002	207	C11 H16 O5	−1.75
Quebrachitol	0.635	233.0422 (M + K)+	194.0789	209/226	C7 H14 O6	0.7
Elenaic acid	8.232	243.0864 (M + H)+	242.0791	225	C11 H14 O6	−0.34
(+)-cis-5,6-Dihydro-5-hydroxy-4-methoxy-6-(2-phenylethyl)-2H-pyran-2-one	8.82	249.112 (M + H)+	248.1048	219/237	C14 H16 O4	0.38
Pyriculol	8.403	249.1124 (M + H)+	248.1051	219	C14 H16 O4	−0.81
D-1-[(3-Carboxypropyl)amino]-1-deoxyfructose	0.645	266.1238 (M + H)+	265.1162	239/247/258	C10 H19 N O7	−0.24

Table 3. *Cont.*

Compound	ts (min)	*m/z* Experimental	Teoric Mass	MS/MS Fragments	Molecular Formula	Error (ppm)
Others						
Acetyltryptophanamide	1.021	268.106 (M + Na)+	245.1168	-	C13 H15 N3 O2	−1.6
Modafinil	0.869	274.0902 (M + H)+	273.0834	245/256	C15 H15 N O2 S	−3.92
Ilicifolinoside A	1.29	282.1546 (M + NH$_4$)+	264.1206	253/270	C11 H20 O7	1
Oxaprozin	7.816	311.139 (M + NH$_4$)+	293.1052	289	C18 H15 N O3	0.13
Fluoxetine	9.646	327.1685 (M + NH$_4$)+	309.1347	309/317	C17 H18 F3 N O	−2.13
Epitestosterone	8.194	327.1709 (M + K)+	288.2081	-	C19 H28 O2	2.74
Compound V(S)	8.415	329.1869 (M + H)+	328.1796	303/311	C19 H24 N2 O3	−2.66
N′-Hydroxyneosaxitoxin	0.864	332.1314 (M + H)+	331.1238	314/322	C10 H17 N7 O6	0.75
p,γ-Dihydroxyphenylbutazone	4.044	341.1499 (M + H)+	340.1427	314/325	C19 H20 N2 O4	−1.16
6′-Hydroxyhydrodolasetron; MDL 73492	8.563	343.1659 (M + H)+	342.1586	325/335	C19 H22 N2 O4	−1.8
b-D-Glucopyranosiduronic acid	7.234	344.1343 (M + H)+	343.1271	319/327	C15 H21 N O8	−1.17
2-(4-Allyl-2-methoxyphenoxy)-1-(4-hydroxy-3-methoxyphenyl)-1-propanol	9.671	345.1697 (M + H)+	344.1625	317/327/335	C20 H24 O5	−0.45
5-(6-Hydroxy-3,7-dimethyl-2,7-octadienyloxy)-7-methoxycoumarin	8.976	345.1701 (M + H)+	344.1625	327	C20 H24 O5	−0.42
URB937	4.487	355.1655 (M + H)+	354.1578	325/343	C20 H22 N2 O4	0.38
Methyl-2-alpha-L-fucopyranosyl-beta-D-galactoside	1.309	358.171 (M + NH$_4$)+	340.1364	328/348	C13 H24 O10	1.47
202-791	7.883	359.1349 (M + H)+	358.1285	-	C17 H18 N4 O5	−2.24
Hydroxyisonobilin	8.976	363.1806 (M + H)+	362.1738	333/345/355	C20 H26 O6	−2.49
5-Megastigmen-7-yne-3,9-diol 9-glucoside	9.003	371.2067 (M + H)+	370.1994	345/355	C19 H30 O7	−0.62
Marshmine	7.412	373.1767 (M + NH$_4$)+	355.1426	343/355	C20 H21 N O5	−1.73
(-)-11-nor-9-carboxy-Δ9-THC	7.381	383.1607 (M + K)+	344.1982	355/373	C21 H28 O4	1.64
Lochnerinine	9.582	383.1967 (M + H)+	382.1892	352/363/371	C22 H26 N2 O4	0.04
Monotropein	7.342	408.1508 (M + NH$_4$)+	390.1169	-	C16 H22 O11	−1.64
Todatriol glucoside	6.91	408.1872 (M + NH$_4$)+	390.1531	377/386/394	C17 H26 O10	−1.4

Table 3. *Cont.*

Compound	ts (min)	*m/z* Experimental	Teoric Mass	MS/MS Fragments	Molecular Formula	Error (ppm)
Others						
Dicaffeoylputrescine	7.271	413.1714 (M + H)+	412.1639	383/391/408	C22 H24 N2 O6	−1.03
Gardenoside	8.232	422.1664 (M + NH$_4$)+	404.1326	243	C17 H24 O11	−1.8
Ganoderol A	18.782	439.3566 (M + H)+	438.3492	411	C30 H46 O2	1.4
Gln Tyr Tyr	9.281	490.2294 (M + NH$_4$)+	472.1955	476	C23 H28 N4 O7	0.68
Trilobolide	8.331	561.2093 (M + K)+	522.2464	534	C27 H38 O10	0.09
Coproporphyrin	8.51	699.2768 (M + K)+	660.3137	243	C36 H44 N4 O8	3.35
Betulinic Acid	18.791	935.7075 (2M + Na)+	456.3596	479/758	C30 H48 O3	1.74

Table 4. Phytochemical profile of the aqueous extract of *A. angustiloba* leaves by LC–MS in the negative ion mode.

Compound	ts (min)	*m/z* Experimental	Teoric Mass	MS/MS Fragments	Molecular Formula	Error (ppm)
Phenolic acids						
3,4-Dihydroxybenzoic acid	3.227	153.0197 (M − H)−	154.027	-	C7 H6 O4	−2.54
2,4,6-Trihydroxybenzoic acid	1.574	169.0146 (M − H)−	170.0218	-	C7 H6 O5	−1.84
Cis-5-Caffeoylquinic acid	7.296	353.0893 (M − H)−	354.0966	-	C16 H18 O9	−4.2
Flavonoids						
6-Hydroxyluteolin 5-rhamnoside	9.323	447.0959 (M − H)−	448.1026	-	C21 H20 O11	−4.48
Apigenin 7-(2″-E-p-coumaroylglucoside	7.091	577.1382 (M − H)−	578.1452	-	C30 H26 O12	−4.87
Robinetin 3-rutinoside	8.73	609.1486 (M − H)−	610.1559	581/593	C27 H30 O16	−4.1
Robinetinidol-(4alpha->8)-catechin-(6->4alpha)-robinetinidol	7.54	865.2001 (M − H)−	866.2069	576/720	C45 H38 O18	−1.22
Glycosides						
(7′R)-(+)-Lyoniresinol 9′-glucoside	8.411	581.2264 (M − H)−	582.2328	-	C28 H38 O13	−2.74
Fatty acids						
11-hydroperoxy-12,13-epoxy-9-octadecenoic acid	11.016	327.2192 (M − H)−	328.2264	309	C18 H32 O5	−4.48
Others						
Oxaloglutarate	0.759	203.0187 (M − H)−	204.026	179/191	C7 H8 O7	4.72
9-Aminoacridine	0.635	229.053 (M + Cl)−	194.0836	203/209/215/223	C13 H10 N2	3.89
Asp Trp Gly	6.886	375.1322 (M − H)−	376.1391	-	C17 H20 N4 O6	−2.15
Acetyl-maltose	0.67	383.1207 (M − H)−	384.1278	357/365	C14 H24 O12	−2.73
Trp Asp Glu	7.64	447.1531 (M − H)−	448.1608	416/429	C20 H24 N4 O8	−3.04
1,2,3,4-Tetragalloyl-alpha-D-glucose	8.688	787.1018 (M − H)−	788.1091	463/609/720	C34 H28 O22	−2.41

Table 5. Phytochemical profile of the 60% methanolic extract of *A. angustiloba* leaves by LC–MS in the positive ion mode.

Compound	ts (min)	*m/z* Experimental	Teoric Mass	MS/MS Fragments	Molecular Formula	Error (ppm)
Phenolic acids						
m-Coumaric acid	8.232	165.0542 (M + H)+	164.0472	137/151	C9 H8 O3	1.13
4-(2-hydroxypropoxy)-3,5-dimethyl-Phenol	9.109	197.1171 (M + H)+	196.1099	-	C11 H16 O3	0.14
Haematommic Acid	0.619	219.0271 (M + Na)+	196.038	195/209	C9 H8 O5	−4.29
Sphagnum acid	9.011	223.0599 (M + H)+	222.0526	197/209	C11 H10 O5	0.86
cis-Sinapic acid	8.231	225.0756 (M + H)+	224.0684	197/211	C11 H12 O5	0.54
1-O-Caffeoylquinic acid	7.304	355.1029 (M + H)+	354.0957	327/343	C16 H18 O9	−1.71
Flavonoids						
5,7,2′,3′-Tetrahydroxyflavone	9.75	287.0552 (M + H)+	286.0479	273	C15 H10 O6	−0.39
Oritin-4beta-ol	7.319	291.0866 (M + H)+	290.0794	262	C15 H14 O6	−1.13
3,5,7,2′,5′-Pentahydroxyflavone	9.325	303.0501 (M + H)+	302.0428	287/295	C15 H10 O7	−0.37
2′,4′,6′-Trihydroxy-3′-prenyldihydrochalcone	8.975	327.1597 (M + H)+	326.1524	303	C20 H22 O4	−1.96
Isovitexin	9.752	433.1135 (M + H)+	432.1063	325	C21 H20 O10	−1.45
6-C-Galactosylisoscutellarein	9.178	449.1081 (M + H)+	448.1011	436	C21 H20 O11	−1.29
6-Hydroxyluteolin 5-rhamnoside	9.325	449.1086 (M + H)+	448.1012	-	C21 H20 O11	−1.39
5,6,7,3′,4′-Pentahydroxy-8-methoxyflavone 7-apioside	8.887	465.1037 (M + H)+	464.0965	341	C21 H20 O12	−2.18
2′,4′,6′,3-Tetrahydroxy-3′-geranyl-6″,6″-dimethylpyrano-[2″,3″:4,5]dihydrochalcone	7.69	515.2392 (M + Na)+	492.2498	484/497/505	C30 H36 O6	2.83
Apigenin 7-(2″-E-p-coumaroylglucoside	6.93	579.1498 (M + H)+	578.1426	394	C30 H26 O12	−0.22
Isovitexin 7-O-rhamnoside	9.588	579.1717 (M + H)+	578.1643	383	C27 H30 O14	−1.22
Luteolin 7-rhamnosyl(1->6)galactoside	9.213	595.1658 (M + H)+	594.1584	355	C27 H30 O15	0.11
Robinetin 3-rutinoside	8.73	611.1607 (M + H)+	610.1537	595	C27 H30 O16	−0.5
Alkaloids						
Caffeine	7.349	195.0878 (M + H)+	194.0805	171/185	C8 H10 N4 O2	−0.4
O-Desmethylquinidine	8.76	311.1753 (M + H)+	310.168	153/193/249	C19 H22 N2 O2	0.48
Sarpagine	9.879	311.1754 (M + H)+	310.1682	287/299	C19 H22 N2 O2	−0.23

Compound	ts (min)	*m/z* Experimental	Teoric Mass	MS/MS Fragments	Molecular Formula	Error (ppm)
Alkaloids						
Benzosimuline	9.567	323.1755 (M + NH$_4$)+	305.1416	293/303/317	C20 H19 N O2	−0.01
Affinine	9.732	325.191 (M + H)+	324.1836	297/309/317	C20 H24 N2 O2	0.42
Caribine	9.628	327.1701 (M + H)+	326.1628	303/317	C19 H22 N2 O3	0.77
Vinorine	8.589	335.1757 (M + H)+	334.1687	311/329/341	C21 H22 N2 O2	−1.62
Akuammicine	9.416	323.1754 (M + H)+	322.1681	303	C20 H22 N2 O2	0.24
Tabersonine	9.626	337.1914 (M + H)+	336.1841	309/317/327	C21 H24 N2 O2	−0.91
Yohimbic Acid	8.143	341.1864 (M + H)+	340.1791	329	C20 H24 N2 O3	−1.25
3-Hydroxyquinidine	8.76	341.1864 (M + H)+	340.1791	-	C20 H24 N2 O3	−1.1
Rauwolscine	9.52	355.2018 (M + H)+	354.1944	327/337	C21 H26 N2 O3	−0.26
Papaverine	8.016	357.181 (M + NH$_4$)+	339.1463	327/341/351	C20 H21 N O4	2.29
11-Methoxy-vinorine	9.056	365.1863 (M + H)+	364.1796	337/345/355	C22 H24 N2 O3	−2.39
14β-Hydroxyyohimbine	9.846	371.1969 (M + H)+	370.1892	343/352	C21 H26 N2 O4	0.06
Glycosides						
Ethyl beta-D-glucopyranoside	0.696	209.1021 (M + H)+	208.0949	-	C8 H16 O6	−0.95
Blumenol C glucoside	9.222	373.2213 (M + H)+	372.2141	355	C19 H32 O7	1.95
(1RS,2RS)-Guaiacylglycerol 1-glucoside	6.884	394.1711 (M + NH$_4$)+	376.1373	-	C16 H24 O10	−0.89
Benzyl O-[arabinofuranosyl-(1->6)-glucoside]	7.64	420.1869 (M + NH$_4$)+	402.153	392/402	C18 H26 O10	−0.98
Lucuminic acid	8.102	464.1766 (M + NH$_4$)+	446.1429	422/448	C19 H26 O12	−0.98
Fatty acids						
2-Dehydro-3-deoxy-D-xylonate	1.076	166.0716 (M + NH$_4$)+	148.0377	-	C5 H8 O5	−3.64
10-Tridecynoic acid	10.444	211.1688 (M + H)+	210.1612	-	C13 H22 O2	3.5
Palmitic amide	19.089	256.2628 (M + H)+	255.2554	-	C16 H33 N O	3.03
9-keto palmitic acid	11.754	271.2266 (M + H)+	270.2192	253	C16 H30 O3	1.21
9,16-dihydroxy-palmitic acid	11.753	289.2378 (M + H)+	288.2303	271	C16 H32 O4	−0.78
2-Hydroxyhexadecanoic acid	12.276	290.2692 (M + NH$_4$)+	272.2354	274	C16 H32 O3	−0.88
13-methyl-octadecanoic acid	13.93	316.3206 (M + NH$_4$)+	298.2867	295	C19 H38 O2	1.71
11-hydroperoxy-12,13-epoxy-9-octadecenoic acid	11.006	346.2588 (M + NH$_4$)+	328.2248	323/337	C18 H32 O5	0.52

<p align="center">**Table 5.** *Cont.*</p>

Compound	ts (min)	*m/z* Experimental	Teoric Mass	MS/MS Fragments	Molecular Formula	Error (ppm)
Others						
2-Amino-3-methyl-1-butanol	0.626	104.1071 (M + H)+	103.0998	-	C5 H13 N O	−0.93
Valine	0.668	118.086 (M + H)+	117.0785	104	C5 H11 N O2	4.16
Pyroglutamic acid	0.924	130.0496 (M + H)+	129.0424	121	C5 H7 N O3	1.22
2,3,5-Trihydroxytoluene	0.697	141.0543 (M + H)+	140.047	121	C7 H8 O3	2.16
Vinylacetylglycine	1.835	144.0656 (M + H)+	143.0585	121	C6 H9 N O3	−1.66
Methylitaconate	2.86	145.0499 (M + H)+	144.0426	121/133	C6 H8 O4	−2.65
3-Hydroxy-3-methyl-glutaric acid	2.572	163.06 (M + H)+	162.0528	133/143	C6 H10 O5	0.11
(2R,3S)-2,3-Dimethylmalate	2.86	163.0601 (M + H)+	162.0528	-	C6 H10 O5	−0.08
3-tert-Butyl-5-methylcatechol	12.149	181.1222 (M + H)+	180.1149	-	C11 H16 O2	0.89
3,4-Dehydro-6-hydroxymellein	8.869	193.0493 (M + H)+	192.0424	-	C10 H8 O4	−0.55
Valiolone	0.667	193.0703 (M + H)+	192.0632	163/173	C7 H12 O6	0.91
Quebrachitol	0.648	195.0865 (M + H)+	194.0792	-	C7 H14 O6	−0.6
(5alpha,8beta,9beta)-5,9-Epoxy-3,6-megastigmadien-8-ol	10.257	209.1532 (M + H)+	208.1458	-	C13 H20 O2	2.46
5-Hydroxyferulate	7.337	211.0599 (M + H)+	210.0526	185/195	C10 H10 O5	1.14
2-Hydroxy-3-carboxy-6-oxo-7-methylocta-2,4-dienoate	7.34	229.0713 (M + H)+	228.064	211	C10 H12 O6	−2.64
Depdecin	7.841	229.107 (M + H)+	228.0998	207	C11 H16 O5	−0.13
Xestoaminol C	12.175	230.248 (M + H)+	229.2407	203/211/219	C14 H31 N O	−0.59
1-O-Methyl-myo-inositol	0.634	233.0429 (M + K)+	194.0798	209/217/226	C7 H14 O6	−4.01
Elenaic acid	8.231	243.0863 (M + H)+	242.079	225	C11 H14 O6	0.23
C16 Sphinganine	12.112	274.2745 (M + H)+	273.2672	244/255	C16 H35 N O2	−1.57
C17 Sphinganine	12.711	288.2897 (M + H)+	287.2824	272	C17 H37 N O2	0.19
2-(beta-D-Glucosyl)-sn-glycerol	0.655	293.0635 (M + K)+	254.1011	247/266/280	C9 H18 O8	−3.86
2,9-Dimethyl-2,9-diazatricyclo[10.2.2.25,8]-octadeca-5,7,12,14,15,17-hexaene-3,10-diol, 9CI	9.912	299.1757 (M + H)+	298.1684	269/283	C18 H22 N2 O2	−0.93
Phytosphingosine	12.203	318.3005 (M + H)+	317.2933	290	C18 H39 N O3	−0.99
Compound V(S)	8.401	329.1863 (M + H)+	328.1789	-	C19 H24 N2 O3	−0.77
URB597	8.535	339.1702 (M + H)+	338.163	335/309	C20 H22 N2 O3	0.22
Epicainide	9.994	339.2069 (M + H)+	338.1987	311/325	C21 H26 N2 O2	2.05
Phenisopham	8.554	343.1653 (M + H)+	342.158	335	C19 H22 N2 O4	−0.02

Table 5. *Cont.*

Compound	ts (min)	*m/z* Experimental	Teoric Mass	MS/MS Fragments	Molecular Formula	Error (ppm)
Others						
5-(6-Hydroxy-3,7-dimethyl-2,7-octadienyloxy)-7-methoxycoumarin	8.975	345.1704 (M + H)+	344.1628	327	C20 H24 O5	−1.13
2-Pyrrolidinone, 4-(2-morpholinoethyl)-3,3-diphenyl	8.16	351.2072 (M + H)+	350.1998	329/341	C22 H26 N2 O2	−1.18
URB937	9.114	355.1662 (M + H)+	354.1588	337	C20 H22 N2 O4	−2.31
Methyl-2-alpha-L-fucopyranosyl-beta-D-galactoside	1.307	358.1708 (M + NH$_4$)+	340.1367	328/341	C13 H24 O10	0.67
202-791	7.879	359.1352 (M + H)+	358.1285	-	C17 H18 N4 O5	−2.06
Marshmine	7.393	373.1763 (M + NH$_4$)+	355.1425	343/355	C20 H21 N O5	−1.38
Akuammine	9.564	383.1971 (M + H)+	382.1897	-	C22 H26 N2 O4	−1.14
N-stearoyl valine	20.36	384.3477 (M + H)+	383.3403	-	C23 H45 N O3	−0.89
3α,12α-Dihydroxy-5β-chol-8(14)-en-24-oic Acid	21.097	391.2847 (M + H)+	390.2774	371	C24 H38 O4	−1.12
Gardenoside	8.231	405.1392 (M + H)+	404.1324	243	C17 H24 O11	−1.23
Monotropein	7.339	408.1503 (M + NH$_4$)+	390.1165	-	C16 H22 O11	−0.71
Gln Tyr Tyr	9.279	490.2294 (M + NH$_4$)+	472.1954	479	C23 H28 N4 O7	0.89
Mascaroside	8.976	542.26 (M + NH$_4$)+	524.2261	365	C26 H36 O11	−0.66
Pheophorbide a	19.793	593.2762 (M + H)+	592.2688	565	C35 H36 N4 O5	−0.46
Coproporphyrin	8.288	699.2765 (M + K)+	660.3135	329	C36 H44 N4 O8	3.6

Table 6. Phytochemical profile of the 60% methanolic extract of *A. angustiloba* leaves by LC–MS in the negative ion mode.

Compound	ts (min)	*m/z* Experimental	Teoric Mass	MS/MS Fragments	Molecular Formula	Error (ppm)
Phenolic acids						
3,4-Dihydroxybenzoic acid	3.203	153.0196 (M − H)−	154.0269	-	C7 H6 O4	−1.59
2,4,6-Trihydroxybenzoic acid	1.568	169.0148 (M − H)−	170.0221	-	C7 H6 O5	−3.24
1,2-Digalloyl-beta-D-glucopyranose	7.304	483.0794 (M − H)−	484.0867	289/353/389	C20 H20 O14	−2.78
1,3,4-Trigalloyl-beta-D-glucopyranose	7.992	635.0907 (M − H)−	636.0979	393/513/577	C27 H24 O18	−2.57

Table 6. *Cont.*

Compound	ts (min)	*m*/*z* Experimental	Teoric Mass	MS/MS Fragments	Molecular Formula	Error (ppm)
Flavonoids						
4,2′,3′,4′-Tetrahydroxychalcone	11.28	271.0623 (M − H)−	272.0695	241/248/259	C15 H12 O5	−3.68
2,6,3′,4′-Tetrahydroxy-2-benzylcoumaranone	9.814	287.0568 (M − H)−	288.0641	277	C15 H12 O6	−2.61
Epifisetinidol-4alpha-ol	7.303	289.0725 (M − H)−	290.0797	-	C15 H14 O6	−2.39
Isovitexin	9.76	431.0999 (M − H)−	432.1073	-	C21 H20 O10	−3.74
Ent-afzelechin-7-O-beta-D-glucopyranoside	9.625	435.1311 (M − H)−	436.1378	431	C21 H24 O10	−1.93
6-Hydroxyluteolin 5-rhamnoside	9.331	447.0952 (M − H)−	448.1019	-	C21 H20 O11	−2.92
Robinetin 7-glucoside	8.885	463.0903 (M − H)−	464.0976	-	C21 H20 O12	−4.59
Apigenin 7-(3″-p-coumaroylglucoside)	6.921	577.1375 (M − H)−	578.1446	375	C30 H26 O12	−3.84
Isovitexin 7-O-rhamnoside	9.599	577.1579 (M − H)−	578.1647	-	C27 H30 O14	−2
Luteolin 7-rhamnosyl(1->6)galactoside	9.22	593.153 (M − H)−	594.1597	447	C27 H30 O15	−2.11
8-C-Glucosyldiosmetin 4″-O-rhamnopyranoside	9.651	607.1682 (M − H)−	608.1759	431	C28 H32 O15	−2.98
Robinetin 3-rutinoside	8.735	609.1478 (M − H)−	610.1551	463	C27 H30 O16	−2.76
Robinetinidol-(4alpha->8)-catechin-(6->4alpha)-robinetinidol	7.546	865.1987 (M − H)−	866.2058	447/728	C45 H38 O18	0.01
Alkaloids						
1-Methylxanthine	0.645	165.0418 (M − H)−	166.0491	147	C6 H6 N4 O2	−0.41
Enprofylline	0.639	229.0502 (M + Cl)−	194.0809	207/215	C8 H10 N4 O2	−2.89
1,3,7-Trimethyluric acid	0.639	245.0453 (M + Cl)−	210.0764	191/215/229	C8 H10 N4 O3	−5.36
O-Desmethylquinidine	8.76	309.1613 (M − H)−	310.1684	-	C19 H22 N2 O2	−0.88
Glycosides						
Inosine	0.656	267.074 (M − H)−	268.0813	245	C10 H12 N4 O5	−1.99
Isobiflorin	7.293	353.0883 (M − H)−	354.0955	-	C16 H18 O9	−1.07
Ethyl 7-epi-12-hydroxyjasmonate glucoside	9.026	415.1987 (M − H)−	416.2058	389/405	C20 H32 O9	−2.89
Dihydroferulic acid 4-O-glucuronide	8.202	371.1 (M − H)−	372.1072	-	C16 H20 O10	−4.17
Catalposide	9.624	481.1363 (M − H)−	482.1435	461/471	C22 H26 O12	−2.2
Prupaside	8.591	551.2126 (M − H)−	552.22	-	C27 H36 O12	1.31
(7′R)-(+)-Lyoniresinol 9′-glucoside	8.419	581.2255 (M − H)−	582.232	-	C28 H38 O13	−1.32
1-Octen-3-yl glucoside	7.48	289.1667 (M − H)−	290.1739	279	C14 H26 O6	−3.41

Table 6. *Cont.*

Compound	ts (min)	*m*/z Experimental	Teoric Mass	MS/MS Fragments	Molecular Formula	Error (ppm)
Fatty acids						
9,16-dihydroxy-palmitic acid	11.768	287.224 (M − H)−	288.2312	264	C16 H32 O4	−4.08
11-hydroperoxy-12,13-epoxy-9-octadecenoic acid	11.029	327.2189 (M − H)−	328.2261	-	C18 H32 O5	−3.58
5,8,12-trihydroxy-9-octadecenoic acid	11.437	329.2346 (M − H)−	330.2418	301/315	C18 H34 O5	−3.5
Others						
N-Acryloylglycine	0.924	128.0356 (M − H)−	129.0426	112	C5 H7 N O3	−0.41
Glutaconic acid	2.572	129.0195 (M − H)−	130.0268	112/119	C5 H6 O4	−1.33
3-Hydroxypicolinic acid	9.789	138.0198 (M − H)−	139.0271	112/119	C6 H5 N O3	−0.99
2-Propenyl propyl disulfide	1.077	147.0308 (M − H)−	148.0382	133	C6 H12 S2	−1.32
3,4-Dihydroxymandelaldehyde	8.496	167.0347 (M − H)−	168.042	-	C8 H8 O4	1.52
4-O-Methyl-gallate	6.591	183.0301 (M − H)−	184.0374	-	C8 H8 O5	−1.1
Nonic Acid	9.518	187.0978 (M − H)−	188.105	173	C9 H16 O4	−0.99
Glu His	0.867	283.1046 (M − H)−	284.1117	-	C11 H16 N4 O5	1.18
Dyphylline	0.647	289.0708 (M + Cl)−	254.1016	267/281	C10 H14 N4 O4	−0.42
Gingerol	13.127	293.1764 (M − H)−	294.1836	-	C17 H26 O4	−1.78
1-Pyrenylsulfate	6.603	297.0238 (M − H)−	298.0312	289	C16 H10 O4 S	−4.07
Histidinyl-Glutamate	0.644	319.0819 (M + Cl)−	284.1129	289/305	C11 H16 N4 O5	−2.88
beta-Glucogallin	1.125	331.0687 (M − H)−	332.0758	-	C13 H16 O10	−4.35
Hydroxyisonobilin	9.678	361.1671 (M − H)−	362.1743	-	C20 H26 O6	−3.87
2′,3′,5′-triacetyl-5-Azacytidine	0.691	369.1065 (M − H)−	370.1137	339/349/365	C14 H18 N4 O8	−3.41
Trp Asp Gly	6.883	375.1306 (M − H)−	376.1383	-	C17 H20 N4 O6	0.09
Monotropein	7.335	389.1107 (M − H)−	390.1175	-	C16 H22 O11	−3.38
Gardenoside	8.234	403.1263 (M − H)−	404.1333	377	C17 H24 O11	−3.68
Trp Gly Phe	9.677	407.173 (M − H)−	408.1805	380/397	C22 H24 N4 O4	−1.79
Trp Thr Ile	9.232	417.2144 (M − H)−	418.2216	405	C21 H30 N4 O5	0.06
Val Trp Glu	7.778	431.1936 (M − H)−	432.2012	403/420	C21 H28 N4 O6	−0.84
Trp Asp Glu	7.643	447.1523 (M − H)−	448.1602	419/429	C20 H24 N4 O8	−1.67
1,2,3,4,6-Pentakis-O-galloyl-beta-D-glucose	8.998	469.0542 (M − 2H) − 2	940.1227	463	C41 H32 O26	−4.82

Table 6. *Cont.*

Compound	ts (min)	*m/z* Experimental	Teoric Mass	MS/MS Fragments	Molecular Formula	Error (ppm)
3-(4-Hydroxy-3-methoxyphenyl)-1,2-propanediol 2-O-(galloyl-glucoside)	7.708	511.1472 (M − H)−	512.1547	501	C23 H28 O13	−3.37
Gibberellin A38 glucosyl ester	8.467	523.2198 (M − H)−	524.2271	509	C26 H36 O11	−2.53
Mascaroside	8.984	523.2211 (M − H)−	524.2274	-	C26 H36 O11	−3.05
Citrusin B	8.537	567.2072 (M − H)−	568.2142	551	C27 H36 O13	2.52
1,2,3,4-Tetragalloyl-alpha-D-glucose	8.683	787.1015 (M − H)−	788.1087	463/609	C34 H28 O22	−1.9

LC–MS Q-TOF was used to screen and identify bioactive chemicals from both extracts, which is essential because it can provide much more trustworthy and legitimate data for using these bioactive chemicals in the human diet to cure a variety of diseases.

A total of 103 active chemicals have been found in aqueous extracts, including several classes, such as alkaloids, tannins, flavonoids, glycosides and phenols, whereas 140 active chemicals were detected in the 60% methanolic extract. The most common phenolic type is flavonoids, with 13 and 20 of them being identified in the aqueous and 60% methanolic extracts, respectively. In contrast, ten and nine phenolic acids were detected in the aqueous and 60% methanolic extracts, respectively.

Gou et al. (2021) stated that indole alkaloids from *Alstonia scholaris*, the same genus as *A. angustiloba*, could be a novel natural treatment for lung-related diseases. Indole alkaloids extracted from this plant can produce long-lasting beneficial effects on individuals who have asthma, acute tracheal bronchitis and post-infection cough [46]. Another study was conducted in which indole alkaloids from *A. scholaris* were reported to lower the percentage of neutrophils and C-reactive protein expression in mice with LPS-induced post-infectious cough [47]. Furthermore, it is effective to be used as a curing agent to treat emphysema [48] and pulmonary fibrosis [49]. LC–MS profiles of *A. angustiloba* demonstrated that this plant is also rich in indole alkaloids, such as sarpagine, yohimbic acid, 14β-Hydroxyyohimbine, vinocrine, akuammicine, tabersonine, rauwolscine and 11-methoxy-vinorine. Yohimbic acid is one of the hypothesised small molecules that could inhibit the expression of aortic valve calcification (AVC)-related genes [50].

Moreover, LC–MS screening of *A. angustiloba* leaves extract exhibited the presence of betulinic acid. Betulinic acid is classified as triterpenoid pentacyclic. Additionally, betulinic acid was successfully purified from *Alstonia boonei*, which hinders folate biosynthesis in malarial *Plasmodium* and promotes mitochondrial pore opening and F1F0 ATPase activity in mice [43]. Wong et al. (2014) reported the presence of 3-*O*-caffeoylquinic acid, 4-*O*-caffeoylquinic acid and 5-*O*-caffeoylquinic acid in the leaves extract of *A. angustiloba* [15]. In contrast, our study has detected cis-5-caffeoylquinic acid in the aqueous extract of *A. angustiloba* leaves and 1-*O*-caffeoylquinic acid in the 60% methanolic extract.

Long-term consumption of polyphenol-rich foods confers numerous benefits, including protection against type 2 diabetes, cardiovascular and neurological illnesses, pancreatitis, osteoporosis, lung damage, cancer and gastrointestinal disorders [42]. The formation of phenoxyl radicals by the phenolic groups of polyphenols accepting an electron causes a beneficial perturbation in cellular oxidative chain reactions. Additionally, polyphenols in food and drinks elevate plasma antioxidant activity due to their accumulation in plasma with endogenous antioxidants, which aids in iron absorption as a pro-oxidative dietary component [51]. Therefore, a negative association exists between the consumption of polyphenol-rich foods and the risk of developing chronic human diseases.

4. Conclusions

In summary, 60% methanolic extract of *A. angustiloba* leaves demonstrated stronger antioxidant activity (lower EC_{50}) than that of aqueous extract, in which the EC_{50} from DPPH was 80.38 and 94.11 µg/mL, while the EC_{50} for the ABTS assay was 85.80 and 115.43 µg/mL, respectively, with higher phenolics and flavonoids contents. The study implies that methanol is the optimal solvent for extracting bioactive compounds from *A. angustiloba* leaves. Nevertheless, both extracts have great potential to be agents for treating various diseases based on the presence of promising bioactive compounds. Therefore, future research should be carried out to explain this plant's specific mechanism of anticancer, antioxidant and other medicinal values. Moreover, the safety profile of this plant must be investigated thoroughly via cytotoxicity, in vivo toxicity and mutagenicity. Extensive investigations should be advanced to clinical settings that can aid pharmaceutical development in the realm of illness treatment and prevention.

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